## REMARKS

### I. Status Summary

Claims 44-55 are pending in the current application. Claims 44-55 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contentions that these claims failed to comply with the written description and enablement requirements of 35 U.S.C. § 112, first paragraph. Claims 44-55 have been subjected to a series of rejections under 35 U.S.C. § 112, second paragraph, upon the contention that the claims are indefinite.

A series of rejections under 35 U.S.C. § 102 and a series of obviousness type double-patenting rejections have been presented based on the following documents: Allioli (1994, *Devel Biol*, 165:30-37; hereinafter "Allioli"); Chang (1995, *Cell Biol. Intl*, Vol 19, pg. 143-149; hereinafter "Chang 1995"); Chang (1997, *Cell Biol Intl*, 21:495-499; hereinafter "Chang 1997"); U.S. Patent No. 5,340,740 (hereinafter "the '740 Patent"); U.S. Patent No. 5,656,479 (hereinafter "the '479 Patent"); U.S. Patent No. 5,840,510 (hereinafter "the '510 Patent"); U.S. Patent No. 6,156,569 (hereinafter "the '569 Patent"); and U.S. Patent Application Serial No. 09/094,176 (hereinafter "the '176 Application").

Claims 45-46 and 49-50 have been canceled without prejudice. Applicants reserve the right to file one or more continuation applications claiming the subject matter encompassed by claims 45-46 and 49-50. Claims 44 and 52-54 have been amended. Support for the amendments can be found throughout the specification of the application as filed, particularly in the claims. Additional support for the amendment to claim 44 can be found on page 10, lines 23-24. Additional support for the amendment to claim 52 can be found on page 13, line 18-19. The amendments to claim 52 is solely for the purpose of correcting typographical errors wherein "conditioned media" mistakenly appears as "condition media" and "stem cell factor (SCF)" mistakenly appears as "stromal cell factor (SCF)". The amendments to claims 53 and 54 are solely for the purpose of clarifying the subject matter encompassed by the claim. None of the amendments presented herein are to be interpreted as a surrender of any subject matter. No new matter has been added by virtue of the

claim amendments. Reconsideration of the application as amended and based on the remarks set forth below is respectfully requested.

II. The Presently Claimed Subject Matter

The presently claimed subject matter relates to a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype. The presently claimed subject matter is based on the inventors' observation that certain cells, when isolated from an avian embryo, can give rise to undifferentiated cells. Thus, the presently claimed subject matter relates to both the isolation of the appropriate cells and the culture to provide cells in an undifferentiated state.

With regard to the appropriate cells, primordial germ cells (PGCs) can be isolated and cultured to form undifferentiated avian cells expressing an embryonic stem cell phenotype. During the isolation procedure, a significant number of stromal cells are isolated along with the PGCs (see Specification at page 8, lines 12-14). Thus, when the cell suspension comprising the PGCs is put into culture, the cell suspension comprises both PGCs and stromal cells.

These cells can be isolated from any avian species and from any tissue wherein PGCs are found. In alternative embodiments of the present invention, PGCs are found in the embryonic gonad or the genital ridge (see Specification at page 10, lines 22-24, and at page 11, lines 2-5). Thus, the presently claimed sustained culture comprises cells isolated from the embryonic gonad or genital ridge, and the isolated cells include both PGCs and stromal cells.

The cells can be isolated from an embryo that is later than stage 14 on the Hamburger & Hamilton staging system, an art-recognized system for characterizing the stage of development of avian embryos. For example, the avian cells can be collected from an embryo later than stage 14 (H&H), from a stage 14 to stage 45 embryo, from a stage 15 to stage 31 embryo, and from a stage 27 to stage 30 embryo. See Specification at page 10, lines 3-8.

Once the cells are isolated, a single cell suspension can be prepared. See Specification at page 10, lines 16-17. This suspension is then deposited in culture

under conditions sufficient to produce an avian cell culture comprising undifferentiated avian cells expressing an embryonic stem cell phenotype. See Specification at page 3, line 19, through page 4, line 2. The conditions can be provided by using a feeder matrix (in one embodiment a preconditioned feeder matrix; see Specification at page 11, line 11, through page 14, line 7; particularly page 13, line 21, through page 14, line 4) and conditioned medium (see Specification page 13, lines 9-23).

The nature of the feeder matrix is such that numerous cell types can be employed in producing it. For example, mouse fibroblast cells can be used, as can cells from other murine species or avian species (see Specification at page 12, lines 3-9). In one embodiment, the feeder matrix is provided by the organ or tissue in which the primordial germ cells are located (for example, the gonad or genital ridge). See Specification at page 11, lines 13-15.

The media that can be used can be any suitable medium, including conditioned medium. This can include BRL conditioned medium and media supplemented with various growth factors. See Specification at page 13, lines 9-20.

Once the cells are isolated and the feeder matrix prepared, the cells comprising the PGCs and stromal cells are seeded onto the feeder matrix (for example, a preconditioned feeder matrix) with media (for example, conditioned medium), and the cells are allowed to form colonies of cells expressing an embryonic stem cell phenotype. These cells can be cultured for at least one or two months. (see Specification at page 13, line 21, through page 14, line 5), which is typical of primary cell cultures and which is significantly greater than the usual two week life of primary cultures of cells from an unincubated avian embryo (see Specification page 14, lines 4-7).

Summarily, in one representative embodiment the presently claimed subject matter relates to a culture of avian cells, wherein the culture is produced by depositing cells (PGCs and stromal cells) isolated from the embryonic gonad or genital ridge of an at least stage 14 avian embryo onto a preconditioned feeder matrix. The cells are then grown in conditioned medium to produce undifferentiated

avian cells expressing an embryonic stem cell phenotype (namely, undifferentiated avian cells having a large nucleus, a prominent nucleolus, and little cytoplasm, the morphological hallmarks of embryonic stem cells).

Applicants wish to note that the presently claimed subject matter relates to the culture as a whole and not just to the undifferentiated avian cells. Thus, the presently claimed subject matter relates to a combination comprising (a) a preconditioned feeder matrix; (b) conditioned medium; and (c) avian primordial germ cells and avian stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from an avian embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system and grown in the sustained culture to produce undifferentiated avian cells expressing an embryonic stem cell phenotype.

## III. Response to the Rejections under 35 U.S.C. § 112, First Paragraph

### A. Response to the Written Description Rejections

Claims 44-55 have been rejected on several bases under 35 U.S.C. § 112, first paragraph, upon the contention that the subject matter was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. According to the United States Patent and Trademark Office (hereinafter "the Patent Office"), the following elements were not contemplated by the specification: culturing avian PGCs for one or two months; isolating PGCs and stromal cells at the same time from avian embryos after stage 14 combined with a "preconditioned feeder matrix"; and culturing avian feeder cells isolated from the gonad/genital ridge with PGCs isolated from embryos later than stage 14. The Patent Office further asserts that the specification does not teach culture conditions required to maintain PGCs in culture for one or two months in the presence of avian feeder cells isolated from the gonad/genital ridge. After careful consideration of the rejections and the Patent Office's bases for the rejections, applicants respectfully traverse the rejections and submit the following.

The Patent Office first asserts that the specification does not teach combining PGCs, stromal cells, and a preconditioned feeder matrix. Applicants respectfully disagree. Claim 44, for example, recites a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype. The culture comprises a combination of a preconditioned feeder matrix, conditioned medium, and avian PGCs and stromal cells, wherein the PGCs and stromal cells are isolated together from an avian embryo later than stage 14 according to the Hamburger & Hamilton (hereinafter "H&H") staging system. Applicants respectfully submit that the specification as filed clearly discloses each of these elements, and further discloses that the elements can be combined.

More specifically, the specification at pages 3-4 under the heading "Summary of the Invention", explicitly discloses the following: "(a) collecting avian cells comprising primordial germ cells from an avian embryo after formation of the primitive streak; (b) depositing the avian cells in contact with a preconditioned feeder matrix; and (c) growing the avian cells on the feeder matrix in the presence of media for a time sufficient to produce an avian cell culture consisting essentially of undifferentiated avian cells expressing an embryonic stem cell phenotype". See Specification, page 3, line 19, through page 4, line 2. Thus, the specification teaches avian PGCs grown on a preconditioned feeder matrix. Further, the specification at page 8, lines 12-14, states that "the avian gonadal cells comprising [PGCs] isolated in accordance with the present invention also comprise a significant number of stromal cells". Thus, the "avian cells comprising primordial germ cells from an avian embryo after formation of the primitive streak" (step (a) above) that are deposited in step (b) above on the preconditioned feeder matrix comprise PGCs and stromal cells. Thus, contrary to the Patent Office's assertion, applicants respectfully submit that the specification explicitly discloses culturing PGCs and stromal cells on a preconditioned feeder matrix. Furthermore, contrary to the Patent Office's contention, the specification does not "teach away from isolating PGCs and stromal cells at the same time combined with a preconditioned feeder matrix" as the process taught in the

specification teaches that the isolation of PGCs as disclosed results in the co-isolation of stromal cells.

Similarly, the Patent Office next asserts that the specification does not suggest culturing PGCs and stromal cells isolated from an embryo after stage 14. Applicants respectfully submit that the specification explicitly discloses on page 10, lines 3-8, that "avian embryos from which cells are obtained for carrying out the present invention are preferably after stage 14, more preferably stage 14 to stage 45, even more preferably stage 15 to stage 31, including stages 17, 18, 19, 20, and 21, and most preferably stage 27-30 of development on the Hamburger & Hamilton (H&H) staging system". Thus, the PGCs and stromal cells that are isolated are after stage 14. Since the specification discloses culturing PGCs, and stromal cells can be isolated concurrently with the PGCs, and the avian embryos from which the cells are obtained are preferably after stage 14, applicants respectfully submit that the specification as filed clearly discloses culturing PGCs and stromal cells isolated from an embryo after stage 14.

Furthermore, contrary to the Patent Office's contention on page 4 of the Official Action, the citation on page 10, lines 3-8, of the present specification does not "merely state cells may be isolated from an embryo after stage 14 and does not state stromal cells are isolated from an embryo after stage 14 or that PGCs and stromal cells are isolated together from an embryo after stage 14". Applicants respectfully submit that when the citation is considered in context, it is clear that the "cells" that "may be isolated from an embryo after stage 14" are the PGCs, along with their co-isolated stromal cells. Thus, the citation clearly discloses isolating PGCs from an embryo after stage 14. As discussed hereinabove, the specification also discloses that during the isolation of PGCs, "the avian gonadal cells comprising [PGCs] isolated in accordance with the present invention also comprise a significant number of stromal cells". Thus, it is respectfully submitted that the specification explicitly discloses that PGCs and stromal cells are isolated together. See Specification at page 8, lines 12-14.

Applicants respectfully submit that since PGCs and stromal cells are isolated together as per the disclosure on page 8, and that the avian embryos from which cells are obtained (i.e., the PGCs and stromal cells) are preferably after stage 14 are per the disclosure on page 10, the specification clearly and reasonably puts together the essential elements of the claimed invention.

The Patent Office asserts that "Example 2 does not relate to isolating stromal cells from an avian, from an embryo, specifically after stage 14, or together with PGCs as in claim 44". Official Action at page 4. The Example states that "gonadal cells were cultured on STO feeder layers for 3-5 days and stained with anti-SSEA-1". Applicants respectfully submit that the Example clearly states that the gonadal cells included PGCs, and the citation on page 8 clearly states that PGCs and stromal cells are isolated together. Thus, the specification teaches the isolation of PGCs from the gonads or genital ridge, that this isolation results in the co-isolation of stromal cells, and that the preferred source of these cells is an avian embryo after stage 14. All of these elements are disclosed in the specification as filed, and all of the elements are reasonably put together.

Next, the Patent Office states that "Example 2 does not relate to a 'preconditioned feeder matrix' from an avian or from an embryo, specifically after stage 14". Official Action at page 4. Even assuming arguendo that the Patent Office's characterization of Example 2 is accurate, applicants point out that claim 44 is not limited to a preconditioned avian feeder layer. Page 20, lines 15-17, clearly state that "the survival and proliferation of the gonadal PGCs was affected by the quality of the STO feeder layer, the number of STO cells seeded, preconditioning of the STO feeder layer, and the number of gonadal cells initially seeded". Thus, Example 2 explicitly recites the use of a preconditioned feeder layer.

With regard to a preconditioned avian feeder layer, the specification discloses the use of a preconditioned feeder layer and the use of a feeder layer from "the organ or tissue in which the primordial germ cells are located (for example, the gonad or genital ridge). See Specification at page 11, lines 13-15. Thus, the specification discloses the use of an avian feeder layer. Applicants further submit that would have

been clear to the ordinary artisan that an avian feeder layer could be preconditioned. As further disclosed in the Laboratory Examples, a feeder matrix is preconditioned by culturing the feeder matrix by itself for one to two days prior to the depositing of gonadal cells comprising primordial germ cells in contact with the feeder matrix. See Specification at page 11, line 17, through page 12, line 2.

The Patent Office, while conceding that page 8, lines 12-14 of the specification discloses isolating PGCs and stromal cells together, asserts that this citation "does not suggest culturing PGCs and stromal cells on a preconditioned feeder matrix". Official Action at page 3. Applicants reiterate that this citation taken in isolation need not disclose culturing on a preconditioned feeder matrix since the specification as a whole discloses the use of a preconditioned feeder matrix to culture the isolated cells. See e.g., Specification at page 11, lines 17-19, and the description of Figure 2 found at page 6, lines 3-6. Thus, the Patent Office's assertion that the "diversity and disparity between the citations do not lead one of skill to believe that applicants contemplated isolating PGCs and stromal cells at the same time from embryos after stage 14 combined with a 'preconditioned feeder matrix' as claimed" is contrary to the overall disclosure of the specification, which includes the culturing of the "isolated cells" on a preconditioned feeder matrix, and the disclosure that PGCs and stromal cells are isolated together.

The Patent Office next contends that the argument presented in Amendment C at page 7, paragraph 1, is unclear. This paragraph related to the Patent Office's prior assertion that the specification does not support the breadth of combining any avian PGCs and avian stromal cells isolated from an avian embryo. In Amendment C, applicants amended claim 44 to recite a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype, comprising a preconditioned feeder matrix; conditioned medium; and avian primordial germ cells and stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together from an embryo later than stage 14 (H&H) and grown in the sustained culture to produce undifferentiated avian cells expressing an embryonic stem cell phenotype, citing support for this amendment on page 8, lines 12-14. Applicants submit that claim 44

was not intended to recite any avian PGCs in combination with any avian stromal cells. Claim 44 encompasses avian PGCs in combination with stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together, i.e. isolated at the same time from the same organ or tissue.

Even assuming arguendo that this was unclear, applicants submit that this does not amount to a 35 U.S.C. § 112, first paragraph, defect. However, in an effort to facilitate prosecution of the instant application, applicants have amended claim 44 to recite a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype, comprising a preconditioned feeder matrix; conditioned medium; and avian primordial germ cells and stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from an embryo later than stage 14 (H&H) and grown in the sustained culture to produce undifferentiated avian cells expressing an embryonic stem cell phenotype. Support for this amendment can be found on page 8, lines 12-14, in combination with page 11, lines 13-15, and page 10, lines 22-24.

Next, the Patent Office, while conceding that the specification discloses the isolation of PGCs from the embryonic genital ridge or gonad, asserts that the specification does not support the breadth of isolating PGCs and stromal cells together from anywhere within an avian embryo as broadly claimed. While applicants traverse this assertion, in order to expedite prosecution of the instant application, applicants have amended claim 44 to recite a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype, comprising a preconditioned feeder matrix; conditioned medium; and avian primordial germ cells and avian stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from an avian embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system and grown in the sustained culture to produce undifferentiated avian cells expressing an embryonic stem cell phenotype.

Next, the Patent Office asserts that the specification does not teach that stromal cells are isolated from the genital ridge. Applicants understand this rejection

to apply to claim 46. Claims 45 and 46 have been canceled, and these elements amended into claim 44. Thus, applicants address this rejection with regard to amended claim 44. Again, the Patent Office appears to be viewing particular lines of the specification in isolation and not in the context of the application as a whole. The application teaches that PGCs are isolated along with stromal cells as indicated hereinabove. The application further teaches that PGCs can be isolated from the genital ridge. Thus, the application teaches that PGCs and stromal cells can be isolated from the genital ridge. For similar reasons, since the application teaches that PGCs can be isolated from the embryonic gonad or genital ridge, applicants submit that the specification as filed teaches that PGCs and stromal cells can be isolated from the embryonic gonad or genital ridge.

Next, the Patent Office contends that the specification does not support isolating a feeder matrix from the genital ridge (claim 48) or "gonadal cells" (claim 49). See Official Action at page 6. The Patent Office concedes that page 11, lines 13-15 supports a feeder matrix from the gonad. Claim 49 has been canceled, and thus the rejection with respect to claim 9 is believed to have been rendered moot. Applicants wish to point out, however, that with respect to claim 48, the specification clearly states at page 11, lines 13-15 state that "a feeder matrix can be derived from or provided by the organ or tissue in which the primordial germ cells are located, e.g., the gonad". "The gonad" is simply exemplary in this sentence, and is thus not intended to limit the "organ or tissue in which the [PGCs] are located". Page 10, line 22, through page 11, line 2, of the specification states that "prior to the disclosure of the present invention, it was the general view among those of ordinary skill in the art that avian embryonic gonadal cells comprising primordial germ cells, such as may be collected from, for example, the avian embryonic genital ridge or gonad, once the embryo had reached a stage associated with gonadal development, were to terminally differentiate to germ cells only". Applicants respectfully submit that when taken together, these citations support the isolation of a feeder matrix from the embryonic genital ridge or gonad. The Patent Office concedes as much in the next paragraph of the Official Action (see page 6), in which it deems persuasive this

argument. Accordingly, applicants understand that the rejection relating to isolating a feeder matrix from the genital ridge and/or the gonad has been withdrawn.

The Patent Office next contends that "stromal cells and feeder matrix cells are not included in the 'cells isolated in the invention', and that "nowhere does the specification imply the 'preconditioned feeder matrix' cells are isolated from an avian embryo after stage 14 as claimed or part of the 'cells isolated in the invention'." Official Action at pages 6-7. Applicants respectfully traverse this assertion, which is understood to apply to claims 47 and 48. The specification discloses that PGCs and stromal cells are isolated together. The specification also discloses "a feeder matrix can be derived from or provided by the organ or tissue in which the primordial germ cells are located". The specification further teaches that the embryo from which the PGCs are isolated is preferably later than stage 14. If a feeder matrix can be derived from or provided by the organ or tissue in which the PGCs are located, and PGCs can be collected from the embryonic gonad or genital ridge of an embryo after stage 14, then the feeder matrix can be derived from or provided by the embryonic gonad or genital ridge of an embryo after stage 14.

The Patent Office next asserts that page 14, lines 4-7, of the specification "merely suggests culturing avian embryo cells for one or two months and does not explicitly or implicitly suggest that the ES cell phenotype is sustained for one or two months as claimed. It is not readily apparent that applicants thought the ES cell phenotype could be sustained for one to two months as claimed ". Official Action at page 7. Applicants respectfully submit that the full paragraph in which this citation appears is as follows:

> In a preferred embodiment, avian embryonic gonadal cells comprising primordial germ cells from a four to five day incubated avian embryo are seeded onto the preconditioned feeder matrix with conditioned medium, and the avian cells give rise to nests or colonies of cells exhibiting an embryonic stem cell phenotype. Unlike the case with mammalian stem cells, it is currently preferred to have a preconditioned feeder matrix to facilitate the survival and development of avian PGCs into undifferentiated avian cells expressing an ESC phenotype. The avian embryo cells of the present invention can be cultured for at least one or two months as is typical for a primary cell

> culture, which is significantly greater than the usual two week life of primary cultures of cells from an unincubated avian embryo.

Specification at page 13, line 21, to page 14, line 7. The context of this paragraph makes it clear that the culturing of the cells is intended to produce undifferentiated avian cells expressing an ESC phenotype.

Further, according to the Guidelines for Examination of Patent Applications under 35 U.S.C. § 112, ¶1, "Written Description" Requirement (hereinafter "the Written Description Guidelines"), there is a "strong presumption" than an adequate written description of the claimed invention is present when the application is filed. Applicants submit that an unsupported statement such as "it is not readily apparent that applicants thought the ES cell phenotype could be sustained for one or two months" offered by the Patent Office does not suffice to overcome the "strong presumption" of adequacy of the written description.

Summarily, applicants respectfully submit that each aspect of this rejection of claims 44-55 under 35 U.S.C. § 112, first paragraph, has been addressed hereinabove. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims has been rendered moot. Accordingly, applicants respectfully request the withdrawal of the rejection with respect to claims 44, 47, 48, and 51-55, and the allowance of these claims at this time.

B. The Enablement Rejection

Claims 44-55 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contention that while the specification is enabling for a culture comprising PGCs and avian feeder cells, the specification does not reasonably provide enablement for culturing PGCs and avian feeder cells for one or two months. According to the Patent Office, the Ponce de Leon reference (1997, Revista Brasileira de Reproducao Animal, Vol. 21:96-101; hereinafter "Ponce de Leon") taught that LIF, bFGF, IGF, and SCF are required for long term culture of avian PGCs. The Patent Office contends that neither the art nor the specification teaches how to culture avian PGCs in the presence of avian feeder cells for one or two months. The Patent Office further

contends that neither the art nor the specification teaches the stage of isolation and culture conditions required to maintain chicken ES cells for at least a month or two, or the stage and conditions required to obtain ES cells in species other than chickens. The Patent Office thus asserts that it would have required undue experimentation to isolate any avian ES cell other than chicken ES cells or to maintain any ES cell for one or two months as claimed. After careful consideration of the rejection and the Patent Office's bases for the rejection, applicants respectfully traverse the rejection and submit the following remarks.

With respect to the first aspect of the rejection, the cited Ponce de Leon document appears to suggest that LIF, bFGF, IGF, and SCF are required for long term culture of PGCs, but the conditions under which these factors are necessary are not disclosed in any meaningful detail in the cited reference. For example, Ponce de Leon does not disclose what media was used to grow the cells. It also does not teach such conditions as the number of feeders used per culture vessel, the number of PGCs seeded, etc. Thus, applicants respectfully submit that it is not possible to determine from the Ponce de Leon reference under what conditions the use of LIF, bFGF, IGF, and SCF is necessary.

In contrast, applicants respectfully submit that the instant application provides sufficient detail as to relevant stage of isolation and culture conditions that can be employed for long term culture of avian PGCs. Initially, the specification discloses that PGCs can be isolated from the gonad and genital ridge from later than stage 14 (H&H) chickens. This explicitly discloses the stage of isolation. Additionally, Figure 1 depicts the influence of the number of STO feeder cells on PGC growth and maintenance; Figure 2 demonstrates the influence of preconditioning of the feeder matrix; and Figure 3 shows the combined effects of the number of STO cells used and the numbers of gonadal cells initially seeded per well. Applicants respectfully submit that these results, in combination with the other disclosure found within the instant application, provide adequate information for one of ordinary skill in the art to establish and maintain undifferentiated avian cells expressing an embryonic stem cell phenotype from PGCs. Applicants further submit that once undifferentiated avian

cells expressing an embryonic stem cell phenotype are established, these same culture conditions are sufficient to maintain the cells in an undifferentiated state. Applicants therefore respectfully submit that by following the disclosure of the specification, one of ordinary skill in the art would be able to grow and maintain PGCs and avian feeder cells for one or two months without undue experimentation.

With regard to the second aspect of the instant rejection, it is respectfully submitted that the Patent Office has not established that it would have required one of ordinary skill in the art undue experimentation to prepare any undifferentiated avian cell expressing an embryonic stem cell phenotype other than chicken cells. Initially, with regard to the stage of isolation, the specification discloses that the appropriate stage is after stage 14 on the Hamburger & Hamilton staging system. Alternatively, the stage is described as after formation of the primitive streak. Applicants respectfully submit that each of these developmental stages are adequately known to one of ordinary skill in the art, and the applicability of these stages to avian species other than chickens is clearly understood to the ordinary artisan. Thus, applicants respectfully submit that the stage of isolation is explicitly disclosed in the specification, and that this description is equally applicable to other avian species as it is to chickens.

The Patent Office further asserts that the parameters required to obtain undifferentiated avian cells expressing an embryonic stem cell phenotype in non-chicken avians were not within the realm of routine experimentation for one of ordinary skill in the art at the time that the invention was made. However, this does not take into account the teachings of the present invention where indeed undifferentiated avian cells expressing an embryonic stem cell phenotype were produced from chicken embryos. Thus, while the working examples of the present U.S. patent application pertain to chicken embryos, it is respectfully submitted that the guidance provided in the present U.S. patent application as filed would provide for the development of ES cells from other avian species, including turkeys, ducks, geese, quails, and pheasants.

It is further noted that at the time of filing the subject application, there had been disclosure in U.S. Patent No. 5,162,125 to Bosselman et al. that avian embryonic cells can be transformed with a retroviral vector wherein the retroviral vector carries, for example, DNA encoding chicken growth hormone or thymidine kinase. Bosselmen et al. disclose the *in ovo* transformation of avian embryo cells where the egg is opened to reveal the stem cells within, and the embryo stem cells are directly exposed to the retroviral vector. The egg is then closed and the bird is allowed to hatch.

Accordingly, applicants respectfully submit that in view of the recognition in the field represented by Bosselman et al. at the time of filing of the present application that manipulation of avian embryos other than chicken embryos could be accomplished, and in view of the teachings of culturing techniques for undifferentiated avian cells expressing an embryonic stem cell phenotype provided in the present application, applicants respectfully submit that the present claims are in compliance with the enablement standards of 35 U.S.C. § 112, first paragraph. As a result, applicants respectfully request the withdrawal of the rejection of claims 44-55 under 35 U.S.C. § 112, first paragraph. Claims 45-46 and 49-50 have been canceled, and thus the rejection is believed to be rendered moot as to these claims. Applicants thus believe that claims 44, 47, 48, and 51-55 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

IV. Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 44-55 have been rejected on several bases under 35 U.S.C. § 112, second paragraph, upon the contention that certain terms and phrases appearing in the claims are unclear. Applicants have considered the individual rejections and the bases therefor, respectfully traverse the rejections, and submit the following.

The first basis for rejection rests on the Patent Office's assertion that the phrase "undifferentiated avian cells expressing an embryonic stem cell phenotype" is unclear (claim 44). According to the Patent Office, "it is unclear if the cells merely

share a phenotype in common with avian ES cells or if the cells are avian ES cells". Official Action at page 11.

Applicants initially note that the Court of Appeals for the Federal Circuit has repeatedly stated that absolute precision is not required to adequately define the metes and bounds of the claims of a patent application. "Section 112, ¶2, requires only reasonable precision in delineating the bounds of the claimed invention." U.S. v. Telectronics, Inc., 8 U.S.P.Q.2d 1217, 1223 (Fed. Cir. 1988), cert. denied, 490 U.S. 1046 (1989) (citation omitted) (emphasis added). The Court of Appeals for the Federal Circuit has also clarified the test for the definiteness of a claim: "[t]he test for definiteness is whether one skilled in the art would understand the bounds of the claim when read in light of the specification. If the claims read in light of the specification reasonably apprise those skilled in the art of the scope of the invention, § 112 demands no more." Miles Laboratories, Inc. v. Shandon, Inc., 27 U.S.P.Q.2d 1123, 1126 (Fed Cir. 1993), cert. denied 510 U.S. 1100 (1994) (citations omitted).

Applicants respectfully submit that the above-noted phrase is specifically defined in the specification and refers to cells with a certain morphology that the art recognizes as being characteristic of ES cells and ES-like cells: namely, a large nucleus, a prominent nucleolus, and little cytoplasm. See Specification at page 9, lines 5-6. The Patent Office contends that the specification "does not define how large, prominent or little the nucleus, nucleolus and cytoplasm are". The Patent Office appears to be taking each element of the phrase individually. It is believed that the skilled artisan, on the other hand, would understand the phrase as a whole to describe cells with an ES cell-like morphology. As further evidence of the acceptance of this phrase in the art, applicants direct the Patent Office's attention to U.S. Patent Nos. 5,340,740; 5,656,479, and 5,830,510, all of which specifically recite "undifferentiated avian cells expressing an embryonic stem cell phenotype". See also U.S. Patent Nos. 6,545,199 ("The established ESC lines from mouse embryos have a characteristic phenotype consisting of a large nucleus, a prominent nucleolus, and relatively little cytoplasm"; column 1, lines 31-34); and 6,667,166 ("ES-like

morphology is characterized as compact colonies with apparently high nucleus to cytoplasm ratio and prominent nucleoli"; column 12, lines 29-31).

Accordingly, applicants respectfully submit that one of ordinary skill in the art would understand the meaning of the objected to phrase. Therefore, applicants respectfully request the withdrawal of the instant rejection.

Next, the Patent Office has rejected the claims based upon the phrases "preconditioned feeder matrix" and "conditioned medium" in claim 44. According to the Patent Office, "it remains unclear how the 'preconditioned feeder matrix' relates to the 'conditioned medium' as claimed...[because] it is unclear if "conditioned medium" is separate from the "preconditioned feeder matrix". Official Action at page 12. Applicants respectfully submit that a preconditioned feeder matrix and conditioned medium do not necessarily relate to each other, as each can be independently derived. According to the specification,

> by the term "preconditioned" it is meant that the feeder matrix is cultured in the presence of media for a period of time prior to the depositing of gonadal cells comprising primordial germ cells in contact with the feeder matrix, e.g. a time sufficient to initiate and establish production of, for example, growth factors or other factors by the feeder matrix. As disclosed in the Laboratory Examples, a feeder matrix is preconditioned by culturing the feeder matrix by itself for one to two days prior to the depositing of gonadal cells comprising primordial germ cells in contact with the feeder matrix.

Specification at page 11, line 19, through page 12, line 2. Thus, applicants respectfully submit that a "preconditioned feeder matrix" is one that has been cultured in media for one or two days prior to the addition of the PGCs.

Conditioned medium, on the other hand, refers to media that has been prepared using the art-recognized technique of growing certain cell types (for example, Buffalo Rat Liver cells) in the media, removing the media, and transferring it to other cells in culture, which then grow in the media. The media thus acquires substances (for example, growth factors) secreted into the media by the cells that were originally grown in it. There is potential overlap between conditioned medium and a preconditioned feeder matrix, in that when the feeder matrix is grown in media

to produce a preconditioned feeder matrix, any media that is removed during or after the preconditioning step could be accurately referred to as conditioned medium (i.e. media in which cells had been grown). However, applicants wish to stress that the conditioned medium recited in claim 44 does not have to be the medium in which the "preconditioning" of the feeder matrix takes place. For example, the conditioned medium can be BRL-conditioned medium, which is conditioned by growing BRL cells therein.

In summary, the preconditioned feeder matrix and the conditioned medium are each independent elements of claim 44, and although the media in which the feeder matrix cells are grown becomes conditioned medium, it is not necessary for that medium to be the conditioned medium recited in the claim. Accordingly, applicants respectfully submit that this aspect of the instant rejection has been addressed, and respectfully solicit withdrawal of the rejection.

The Patent Office next asserts that it remains unclear how PGCs isolated from an embryo later than stage 14 are distinguished from PGCs isolated from a stage X or a stage 14 embryo. According to the Patent Office, PGCs isolated from stage X, 14, and after stage 14 have the same structure and function.

In an effort to clarify the claim, applicants have amended claim 44 to recite a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype, comprising a preconditioned feeder matrix; conditioned medium; and avian primordial germ cells and avian stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from an avian embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system and grown in the sustained culture to produce undifferentiated avian cells expressing an embryonic stem cell phenotype. Applicants respectfully note that prior to the invention of the present application, it was not believed to be possible to provide a sustained culture of avian cells expressing a pluripotent embryonic stem cell phenotype that could be derived from PGCs isolated from a later than stage 14 embryo. Indeed, it was believed that PGCs

isolated from later than stage 14 embryos were destined for terminal differentiation into germ cells instead of other tissues.

Summarily, applicants respectfully submit that they have clarified the nature of the presently claimed subject matter. As a result, applicants respectfully submit that this aspect of the instant rejection of the claims has been addressed, and request withdrawal of the instant rejection.

Next, the Patent Office has rejected claim 49 on the basis that the term "gonadal cells". Claim 49 has been canceled, and thus the rejection of this claim is believed to have been rendered moot.

Claims 45 and 46 have also been rejected upon the contention that the claims limit from where the stromal cells are derived but that the claim upon which these claims depend recite that stromal cells and PGCs are isolated together. Applicants wish to thank the Examiner for his suggestion for clarifying these claims. However, claims 45 and 46 have been canceled, and thus the rejection of these claims is believed to be rendered moot.

Claims 49 and 50 have been rejected upon the contention that "the avian gonadal cells" and "the avian genital ridge cells" lack antecedent basis, and further that they are substantial duplicates of claims 47 and 48, respectively. Claims 49 and 50 have been canceled, and thus the rejection of these claims is believed to have been rendered moot.

Claims 53 and 54 have been rejected for the use of the term "sustained" in reference to the "embryonic stem cell phenotype". The Patent Office contends that "sustained" means capable of undergoing further cell division, and that a phenotype cannot "undergo further cell division". Applicants respectfully submit that the specification teaches that the term "sustained" as used herein with respect to ES cells and ES cell cultures refers to a cell or cell culture capable of undergoing further cell division, even if the cells are eventually subject to senescence. See Specification at page 9, lines 1-3. However, in an effort to clarify the claims, claims 53 and 54 have been amended to replace the phrase "phenotype is sustained" with "phenotype

is maintained". Applicants respectfully submit that the instant rejection has been addressed, and respectfully request the withdrawal of the rejection.

In summary, applicants respectfully submit that the rejections of claims 44-55 under 35 U.S.C. § 112, second paragraph, have been addressed, and that the claims are in condition for allowance at this time. Claims 45-46 and 49-50 have been canceled, and as a result, applicants respectfully solicit a Notice of Allowance as to claims 44, 47, 48, and 51-55.

## V. Rejections under 35 U.S.C. § 102

### A. Rejection in view of Allioli

The Patent Office has rejected claims 44-55 under 35 U.S.C. § 102(b) upon the contention that the claims are anticipated by Allioli (1994, Devel. Biol. Vol. 165, pages 30-37; hereinafter "Allioli"). According to the Patent Office, the cited reference taught isolating chicken cells from the gonads of stage 27-28 embryos and culturing the cells in media. Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

It is well settled that for a reference to anticipate a claim under 35 U.S.C. § 102(b), the reference must disclose each and every element of the claim. Applicants respectfully submit that Allioli does not anticipate claims 44-55 because Allioli does not disclose each and every element of the claim. As claimed in claim 44, applicants' invention is a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype comprising the following elements: (a) a preconditioned feeder matrix; (b) conditioned medium; and (c) avian primordial germ cells and avian stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from an avian embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system. Applicants respectfully submit that Allioli does not disclose either a preconditioned feeder matrix or conditioned medium as those terms are used in the instant application and understood by those of skill in the art.

Allioli does not disclose the use of conditioned medium, also an element of claim 44. Allioli discloses growing the cells in DMEM/F12 medium containing penn/strep, glutamine, and in some examples, 10% FCS. This medium is not believed to be a conditioned medium, and thus Allioli does not disclose this element of claim 44 either.

Since Allioli does not disclose each and every element of claim 44, applicants respectfully submit that Allioli does not anticipate claim 44. Claims 45-55 all depend directly from claim 44, and thus include all the elements of distinguished claim 44. Accordingly, applicants respectfully request the withdrawal of the rejection of claims 44-55 in view of Allioli. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants therefore respectfully submit that claims 44, 47, 48, and 51-55 have been distinguished from Allioli, and respectfully request a Notice of Allowance to that effect.

B. Rejection in view of Chang 1995

Claims 44-55 have been rejected under 35 U.S.C. § 102(b) upon the contention that the claims are anticipated by Chang (1995, Cell Biol. Internatl., Vol. 19, pages 143-149; hereinafter "Chang 1995"). According to the Patent Office, Chang 1995 taught isolating stromal cells from the genital ridge of stage 27-28 embryos, and culturing the cells in media containing IGF, FGF, and LIF. The Patent Office further asserts that these cells inherently contain PGCs. Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

Applicants respectfully submit that Chang 1995 does not anticipate the instant claims because Chang 1995 does not disclose each and every element of the claims. Specifically, Chang 1995 does not disclose the use of conditioned medium, or a preconditioned feeder matrix in conjunction with PGCs isolated from a later than stage 14 embryo. Rather, Chang 1995 discloses Medium 199 based on Earl's balanced salt solution supplemented with 10% FBS, IGF-1, bFGF, and murine LIF. This is not believed to be a conditioned medium, and thus the cited reference does not disclose each and every element of claim 44.

Since Chang 1995 does not disclose each and every element of claim 44, applicants respectfully submit that Chang 1995 does not anticipate claim 44. Claims 45-55 all depend directly from claim 44, and thus include all the elements of distinguished claim 44. Accordingly, applicants respectfully request the withdrawal of the rejection of claims 44-55 in view of Chang 1995. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants therefore respectfully submit that claims 44, 47, 48, and 51-55 have been distinguished from Chang 1995, and respectfully request a Notice of Allowance to that effect.

C. Rejection in view of Chang 1997

Claims 44-55 have been rejected under 35 U.S.C. § 102(b) upon the contention that the claims are anticipated by Chang (1997, Cell Biol. Internatl., Col. 21, pages 495-499; hereinafter "Chang 1997"). According to the Patent Office, Chang 1997 teaches isolating germinal ridge stromal cells from stage 27-28 embryos, which were then cultured for 5 days in media containing IGF, FGF, and LIF with germinal ridge stromal feeder cells isolated from day 5 embryos to obtain gPGCs. Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

Applicants respectfully submit that Chang 1997 does not anticipate the instant claims because Chang 1997 does not disclose each and every element of the claims. Specifically, Chang 1997 does not disclose the use of conditioned medium. Rather, Chang 1997 employs the same culture conditions as Chang 1995, and thus the discussion above for Chang 1995 is applicable here. Summarily, the medium employed in these references is not believed to be conditioned medium, and thus the cited reference does not disclose each and every element of claim 44.

Since Chang 1997 does not disclose each and every element of claim 44, applicants respectfully submit that Chang 1997 does not anticipate claim 44. Claims 45-55 all depend directly from claim 44, and thus include all the elements of distinguished claim 44. Accordingly, applicants respectfully request the withdrawal of the rejection of claims 44-55 in view of Chang 1997. Claims 45-46 and 49-50 have

been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants therefore respectfully submit that claims 44, 47, 48, and 51-55 have been distinguished from Chang 1997, and respectfully request a Notice of Allowance to that effect.

D. Rejection in view of the Petitte Patents

Claims 44-55 have been rejected under 102(e) as being anticipated by U.S. Patent Nos. 5,340,740; 5,656,470, or 5,840,510 (hereinafter collectively referred to as "the Petitte Patents"). According to the Patent Office, the Petitte Patents teach culturing cells from a stage X embryo and isolating PGCs ('740 Patent). The cells were seeded onto chicken embryonic fibroblast feeder layers and cultured with BRL conditioned medium. The Patent Office asserts that "PGCs isolated from stage X are equivalent to PGCs isolated later than stage 14 as claimed because PGCs isolated from stage X and XIV have the same function". See Official Action at page 19 (emphasis added). Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

Applicants respectfully submit that the Petitte Patents do not, either alone or in conjunction, anticipate the instant claims because the Petitte Patents do not disclose each and every element of the claims. Specifically, the Petitte Patents do not disclose the use of PGCs isolated from the gonad or genital ridge of an avian embryo at a stage later than stage 14.

To reiterate, there are two main art-recognized staging systems, the EGK staging system and the H&H staging system. The former uses Roman numerals, and the latter Arabic numerals. The EGK system generally relates to those stages prior to formation of the primitive streak, whereas the H&H system begins at stage 1 after the late cleavage stage.

The avian embryos disclosed in the Petitte Patents are all staged according to the EGK staging system, wherein stage IX through stage XIV embryos are disclosed. Thus, the Petitte Patents do not disclose the isolation of PGCs from later than stage 14 (H&H) embryos. Additionally, the PGCs of claim 44 are isolated along with

stromal cells from the embryonic gonad or genital ridge, structures that are not present in the stage X or XIV avian embryo.

Since the Petitte Patents do not disclose each and every element of claim 44, applicants respectfully submit that the Petitte Patents do not anticipate claim 44. Claims 45-55 all depend directly from claim 44, and thus include all the elements of distinguished claim 44. Accordingly, applicants respectfully request the withdrawal of the rejection of claims 44-55 in view of the Petitte Patents. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants therefore respectfully submit that claims 44, 47, 48, and 51-55 have been distinguished from the Petitte Patents, and respectfully request a Notice of Allowance to that effect.

E. Rejection in view of U.S. Patent No. 6,156,569

Claims 44-55 have been rejected under 35 U.S.C. § 102(e) upon the contention that the claims are anticipated by U.S. Patent No. 6,156,569 to Ponce de Leon (hereinafter "the '569 Patent"). According to the Patent Office, the '569 Patent teaches isolating PGCs from stage XIV embryos, which were then cultured in complete media containing IGF, FGF, SCF, and LIF for at least 25 days. Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

Applicants respectfully submit that the '569 Patent does not anticipate the instant claims because the '569 Patent does not disclose each and every element of the claims. Specifically, the '569 Patent does not disclose either the use of PGCs isolated from the gonad or genital ridge of an avian embryo at a stage later than stage 14 or the use of conditioned medium.

Initially, applicants respectfully submit that the PGCs disclosed in the '569 Patent were isolated from the embryonic blood of stage 12-14 embryos (see column 5, lines 5-9). Thus, the PGCs disclosed are not PGCs isolated from the gonad or genital ridge of an avian embryo at a stage later than stage 14. Furthermore, the isolated PGCs were grown in "complete medium", which according to the '569 Patent is $\alpha$-MEM supplemented with glutamine, antibiotics, FCS, $\beta$-mercaptoethanol, and

various growth factors. This is not believed to be conditioned medium. Accordingly, the '569 Patent does not disclose each and every element of the instant claims.

Given the lack of disclosure of PGCs isolated from the gonad or genital ridge of an avian embryo at a stage later than stage 14 and the use of conditioned medium, applicants respectfully submit that the cited reference does not anticipate claims 44-55. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants therefore respectfully submit that claims 44, 47, 48, and 51-55 have been distinguished from the '569 Patent, and respectfully request a Notice of Allowance to that effect.

VI. Double Patenting Rejections

A. Rejection based on 09/094,176

Claims 44-55 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 21-27 of copending Application No. 09/094,176 (hereinafter "the '176 Application"). According to the Patent Office, "the conflicting claims are not patentably distinct from each other because the avian PGCs having an ES cell phenotype in the instant application are used in the method of '176". Official Action at page 21. Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

Preliminarily, applicants note that the U.S. Court of Appeals for the Federal Circuit (C.A.F.C.) has set forth in Environmental Design Ltd. v. Union Oil Co., 713 F.2d 693 (Fed. Cir. 1983), cert. denied, 464 U.S. 1043 (1984), that the factual determinations to be made, as well as the evidence to consider, in making an obviousness determination under §103 include:

a) the scope and content of the prior art;

b) the differences between the prior art and the claimed invention;

c) the level of ordinary skill in the pertinent art; and

d) additional evidence, which may serve as indicia of non-obviousness.

All relevant evidence on each of these four dispositive issues must be fully considered and evaluated to determine whether the claimed invention would have been obvious. Additionally, it is well known that for an obviousness-type rejection to stand, the cited document or combination must disclose all aspects of the claimed invention; contain a suggestion to modify the cited document(s) to arrive at the claimed invention; and there must be a reasonable chance of success to reach the claimed invention as a result of the modifications.

In Hodosh v. Block Drug Co., 786 F.2d 1136 (Fed. Cir. 1986), the U.S. Court of Appeals for the Federal Circuit set forth what is described as the "tenets of patent law that must be adhered to when applying §103", Id. at 1143, n.5. Those tenets set out in Hodosh are:

a) the claimed invention must be considered as a whole;

b) the references must be considered as a whole and suggest the desirability and thus obviousness of making the combination;

c) the references must be reviewed without benefit of hindsight vision afforded by the claimed invention; and

d) "ought to be tried" is not the standard with which obviousness is determined.

Applicants submit that the '176 Application does not meet the requirements for a prima facie case of obviousness because the '176 Application does not disclose the use of avian primordial germ cells and avian stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from an avian embryo at a stage later than stage 14.

The Patent Office asserts that the PGCs employed in the combination claimed in claim 44 of the instant application have the same structure and function as those used in the method of the '176 Application. According to the Patent Office, "the stage during which the PGCs are isolated does not bear patentable weight in the instant product claims because the product claimed has the same structure and function as the one used in the method of '176". Official Action at pages 21-22.

Applicant submit, and the Patent Office has acknowledged (see Official Action at page 19), that prior to the present disclosure, it was believed by those of skill in the art that PGCs isolated later than stage 14 (H&H) were incapable of forming pluripotent undifferentiated avian cells expressing an embryonic stem cell phenotype. Indeed, it was believed that PGCs isolated from later than stage 14 embryos were destined for terminal differentiation into germ cells instead of other tissues. Thus, applicants respectfully submit that the art taught away from the use of the instantly claimed cells, that the ability to form undifferentiated avian cells expressing an embryonic stem cell phenotype is an unexpected result.

Accordingly, applicants respectfully submit that a prima facie case of obviousness has not been made out, and as a result, applicants respectfully request that the rejection of claims 44-55 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 21-27 of the '176 Application be withdrawn. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants therefore respectfully submit that claims 44, 47, 48, and 51-55 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

B. Rejection based on U.S. Patent No. 5,340,740 in view of Chang 1995

Claims 44-55 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 8-10 of U.S. Patent No. 5,340,740 (hereinafter "the '740 Patent") in view of Chang 1995. According to the Patent Office:

> Claims 1 and 8-10 claim a sustained culture of undifferentiated avian cells having an ES cell phenotype and methods of making such a culture. '740 did not claim culturing the cells on avian feeder cells or the cell culture made by the method.
>
> However, at the time of filing, Chang taught culturing PGCs on avian stromal cells. Thus, it would have been obvious to one of ordinary skill in the art at the time the invention was made to isolate avian cells having an ES cell phenotype as taught by '740, wherein the avian cells are cultured on avian feeder cells. One of ordinary skill in the art at the time the invention was made would have been motivated to use avian

feeder cells to increase the number of PGCs as taught by Chang (abstract).

Official Action at page 22. Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

Applicants respectfully submit that the combination of the '740 Patent and Chang 1995 does not suffice to create a prima facie case of obviousness. Particularly, each and every element of the presently claimed invention is not disclosed in the cited references, the cited references in fact teach away from the claimed combination, and thus one of ordinary skill in the art would not have been motivated to combine the cited references to arrive at the instantly claimed invention.

Applicants respectfully submit that the claimed invention is a combination of a preconditioned feeder matrix, conditioned medium, and avian primordial germ cells and avian stromal cells, wherein the avian primordial germ cells and stromal cells are isolated together from the embryonic genital ridge or gonad from an avian embryo at a stage later than stage 14 according to the Hamburger & Hamilton staging system and grown in the sustained culture to produce undifferentiated avian cells expressing an embryonic stem cell phenotype. The burden rests on the Patent Office to present evidence that the skilled artisan would be motivated to combine the teachings of the cited references to arrive at the combination claimed. Applicants respectfully submit that it would not be possible to find such motivation as the understanding of the ordinary artisan at the time the application was filed was that PGCs isolated after stage 14 would be incapable of forming the claimed sustained culture.

Thus, applicants respectfully submit that the art taught away from the use of the instantly claimed cells, that the ability to form undifferentiated avian cells expressing an embryonic stem cell phenotype is an unexpected result, and that it is only by viewing the applicants' disclosure with the benefit of hindsight vision that one would recognize that PGCs isolated from the embryonic gonad or genital ridge after stage 14 would form pluripotent undifferentiated avian cells expressing an embryonic stem cell phenotype.

Accordingly, applicants respectfully submit that a prima facie case of obviousness has not been made out, and as a result, applicants respectfully request that the rejection of claims 44-55 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 8-10 of the '740 Patent in view of Chang 1995 be withdrawn. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants therefore respectfully submit that claims 44, 47, 48, and 51-55 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

C. Rejection based on U.S. Patent No. 5,656,470 or 5,830,510 in view of Chang 1995

Claims 44-55 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of U.S. Patent No. 5,656,470 or 5,830,510 (hereinafter "the '470 Patent" and "the '510 Patent", respectively) in view of Chang 1995. Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

Applicants initially note that the discussion presented immediately above with regard to the rejection of claims 44-55 as obvious over the '740 Patent in view of Chang 1995 is applicable to the instant rejection. Summarily, none of the constituent patents that make up the "Petitte Patents" (i.e. the '740 Patent, the '479 Patent, and the '510 Patent) disclose the use of PGCs isolated from an avian embryo after stage 14. The Patent Office's burden is to demonstrate that one of ordinary skill in the art would have been motivated by the teachings of the cited references to create the claimed combination itself. Applicants have contended, and the Patent Office has acknowledged, that those of skill in the art believed that the instantly claimed cells were believed to be incapable of forming undifferentiated avian cells expressing an embryonic stem cell phenotype. Thus, the art teaches away from the production of the instantly claimed combination, and thus, there could have been no motivation to employ PGCs isolated from after stage 14.

Accordingly, applicants respectfully submit that a prima facie case of obviousness has not been made out, and as a result, applicants respectfully request that the rejection of claims 44-55 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of either the '479 Patent or the '510 Patent in view of Chang 1995 be withdrawn. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants therefore respectfully submit that claims 44, 47, 48, and 51-55 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

D. Rejection based on U.S. Patent No. 6,156,659 in view of Chang 1995

Claims 44-55 have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-12 of U.S. Patent No. 6,156,569 (hereinafter "the '569 Patent") in view of Chang 1995. Applicants have carefully considered the rejection and the Patent Office's bases therefor, and respectfully traverse the rejection as follows.

The '569 Patent does not disclose the use of PGCs isolated from an avian embryo after stage 14. The Patent Office's burden is to demonstrate that one of ordinary skill in the art would have been motivated by the teachings of the cited references to create the claimed combination itself. The applicants have contended, and the Patent Office has acknowledged, that those of skill in the art believed that the instantly claimed cells were believed to be incapable of forming undifferentiated avian cells expressing an embryonic stem cell phenotype. Thus, the art teaches away from the production of the instantly claimed combination. Thus, there could have been no motivation to employ PGCs isolated from after stage 14.

Accordingly, applicants respectfully submit that a prima facie case of obviousness has not been made out, and as a result, applicants respectfully request that the rejection of claims 44-55 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-12 of the '569 Patent in view of Chang 1995 be withdrawn. Claims 45-46 and 49-50 have been canceled, and thus the rejection as to these claims is believed to be rendered moot. Applicants

therefore respectfully submit that claims 44, 47, 48, and 51-55 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

## CONCLUSION

In light of the above Amendments and Remarks it is respectfully submitted that the present application is now in proper condition for allowance, and such action is earnestly solicited.

If any minor issues should remain outstanding after the Examiner has had an opportunity to study the Amendment and Remarks, it is respectfully requested that the Examiner telephone the undersigned attorney so that all such matters may be resolved and the application placed in condition for allowance without the necessity for another Action and/or Amendment.

## DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any deficiencies of payment or credit any overpayments associated with the filing of this Amendment After Final to Deposit Account No. **50-0426**.

Respectfully submitted,

JENKINS, WILSON & TAYLOR, P.A.

Date: January 2, 2004 By: _____

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297/93/2 AAT/CPP/ptw